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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/505,252	08/19/2004	Francois Romagne	INN-112	7415

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EXAMINER	
FORD, ALLISON M	
ART UNIT	**PAPER NUMBER**
1651	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	02/08/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/505,252	ROMAGNE ET AL.	
	Examiner	Art Unit	
	Allison M. Ford	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 October 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *21,23-25 and 27-52* is/are pending in the application.

4a) Of the above claim(s) *35-50* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *21,23-25,27-34,51 and 52* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants response of 10 October 2006 has been received and entered into the case. Claims 1-20, 22 and 26 are cancelled. Claims 21 and 32 are amended. Claims 51 and 52 are added as new. Claims 21, 23-25 and 27-52 are pending in the current application, with claims 35-50 being withdrawn from consideration as being directed to a non-elected invention.

Response to Arguments

Applicants' arguments received with the response of 10 October 2006 have been fully considered, and are persuasive in part. Each argument is addressed below, as appropriate. Rejections/objections not repeated below have been withdrawn from consideration.

Regarding the rejection of claims 21-34 under 35 USC 112, first paragraph, as lacking sufficient written description to show possession of (i) the entire genus of 'synthetic activator compounds' of gamma delta T cells, and (ii) the entire genus of cytokines useful in the current application, applicants argue that the as-filed specification did provide sufficient disclosure of a representative number of species of each genus, and also provided teachings and directions for one of ordinary skill in the art to identify further species. However, applicants have narrowed the scope of independent claim 21 to specifically state the synthetic activator compound of gamma delta T lymphocytes is selected from phsophohalohydrins, phosphoepoxides, pyrophosphates, biphosphonates or bisphosphonates. The specification does disclose a sufficient number of species of each of these types of synthetic activator compounds, as well as the common structure and function of each type of molecule; therefore applicants do have written description for the narrowed scope of the claim. Independent claim 21 has also been narrowed to be directed to use of cytokines IL-2 or IL-15. The narrowed scope of claim 21 has obviated the rejection based on lack of written description, the rejection is withdrawn.

Regarding the rejection of claims 21-34 under 35 USC 112, first paragraph, as lacking enablement for performing the claimed method on any biological preparation, applicants have narrowed the scope of independent claim 21 to specifically require the biological preparation to be a blood sample or a cytapheresis sample, both of which are supported and enabled by the specification as-filed. Therefore the rejection is withdrawn.

Regarding the rejection of claims 21-34 under 35 USC 103(a), as being unpatentable over Belmant et al (WO 00/12516) or Espinosa et al (Journal of Biological Chemistry, 2001), each in view of Garcia et al (Journal of Immunology, 1998) and Valeri (Blood Banking and the Use of Frozen Blood Products, 1976), applicants argue that the combination of references fails to teach each and every limitation of the presently claimed invention, specifically the combination of teachings fails to teach or suggest maintaining the cells undergoing culture at a density less than about 5×10^6 cells/mL during said culture step. Furthermore, applicants' assert that maintenance of the cell culture at a concentration less than about 5×10^6 cells/mL results in unexpected results, specifically that when the density was maintained at such levels, greater than about 90-95% gamma delta T lymphocytes were observed after 10, 15 and 21 days of culture.

In response to applicants' argument that the combination of references does not teach the limitation regarding the cell density, it is maintained that manipulation of culture density is result effective variable that is routinely optimized by the skilled artisan. The density at which a cell culture can be maintained clearly has an upper limit (at which point the cells would die from lack of substrate to adhere to, lack of oxygen, too great concentration of waste products, all results of overcrowding); therefore elucidation of the optimal cell density would be a matter of routine experimentation. It is further noted that the density at which the cells can be maintained is also effected by how often the cells

are passage and how long the culture is to be maintained. Therefore, though the cited references do not specifically teach maintaining the culture at a density of less than 5 x 10^6 cells/mL, it is maintained that determination of a successful cell density would have been well within the purview of the skilled artisan at the time the invention was made.

In response to applicants' argument of unexpected results (greater, more consistent concentrations of gamma delta T lymphocytes in the final cell culture) when the cells were maintained at a density of less than 5 x 10^6 cells/mL, it is noted that the results on which applicant relies (specifically Example IIA & Table 8) do not show that the concentration of gamma delta T lymphocytes varies with changing cell densities. Rather the results show that cultures maintained at three different cell densities: 2 x 10^5 cells/mL, 5 x 10^5 cells/mL, 1.5 x 10^6cells/mL, all achieved a gamma delta T lymphocyte population of 94%; these results, while sufficient to show operability of the method, does not provide evidence that the same cell cultures, when maintained at cell densities greater than 5 x 10^6 cell/mL were incapable of achieving such gamma delta T lymphocyte populations. Though applicants point to the results of Belmant et al and Espinosa et al, they fail to particularly point out which specific results or experiments they are relying upon to show inconsistencies. Neither Belmant et al nor Espinosa et al were asserted to anticipate the claimed invention, rather, the rejection is based on the obviousness of the instant invention, including the culture parameters (e.g. cell density), over the cited references.

It is further noted that, with the exception of claim 34, none of the claims require the method produce a cell population that is at least 80% gamma delta T lymphocytes.

Regarding the provisional obviousness-type double patenting rejection of claims 21 and 32 over copending application no. 10/537,394 (claims 80 and 100), it is noted that the amendment to the current independent claim differentiates the instant method from that of the co-pending application, and therefore the rejection of record is withdrawn.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 21, 23-25, 27-34 and 51-52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Belmant et al (WO 00/12516 (French Language); US Patent 6,660,723, which is the US national stage application, is relied upon as a direct translation), in view of Garcia et al (Journal of Immunology, 1998) and Valeri (Blood Banking and the Use of Frozen Blood Products, 1976).

Applicants' claims are directed to a method for preparing a gamma delta T lymphocyte composition comprising culturing a biological preparation, comprising a blood sample or a cytapheresis sample, comprising at least 50 million mononuclear cells in the presence of (i) a synthetic activator compound of gamma delta T lymphocytes, selected from the group consisting of phosphohalohydrins, phosphoepoxides, pyrophosphates, biphosphonates, and bisphosphophonates; and (ii) a cytokine, selected from IL-2 or IL-15; and maintaining the cells at a density less than about 5×10^6 cells/mL during said culturing step. Claim 23 requires the sample to be from cytapheresis. Claim 24 requires the biological preparation to comprise more than 10×10^7 cells. Claim 25 requires the biological preparation to have previously been frozen. Claim 27 requires the cells to be cultured for greater than or equal to 10 days, claims 28 requires the culture period to be 10 to 25 days. Claims 29-31 require the synthetic activator to be BrHPP. Claim 32 requires the cytokine to be IL-2. Claim 33 requires the cytokine to be used at a concentration of about 150 U/mL to about 500 U/mL. Claim 34 requires the resulting gamma delta T lymphocyte population to comprise at least 80% gamma delta T lymphocytes and for the composition to comprise more than 100 million viable and functional gamma delta T cells. New claim 51 requires the

synthetic activator compound to be BrHPP, and the cytokine to be IL-2. New claim 52 requires the cytokine to be IL-15.

Belmant et al teach a large variety of phosphohalohydrins as well as methods of using the phosphohalohydrins for gamma delta T lymphocyte activation (See Belmant et al, col. 2, ln 44-65). As one of the exemplary phosphohalohydrins Belmant et al teach 3-(bromomethyl)-3-butanol-1-yl-diphosphate (BrHPP) (See Belmant et al, col. 17, ln 30-60) (Claims 29-31).

Belmant et al also teach a method for activation of gamma delta T lymphocytes comprising contacting gamma delta T lymphocytes with a) one of their phosphohalohydrins; and b) interleukin-2 (IL-2). Belmant et al further teach the gamma delta T lymphocytes may be part of a peripheral blood sample or blood extract (See Belmant et al, col. 11, ln 63-col. 12, ln 35 & claim 13) (Claims 32, 51 & 52). Therefore, it is within the scope of Belmant et al to contact a peripheral blood sample with BrHPP and IL-2 in order to activate and expand the gamma delta T lymphocyte population present within the peripheral blood sample.

While Belmant et al merely teach 'contacting' the gamma delta T lymphocytes with the BrHPP and IL-2 in a medium (blood), instead of a specific 'culture' step, it would have been well within the purview of one of ordinary skill in the art at the time the invention was made to interpret the 'contacting' of Belmant et al as a culturing step. The method of Belmant et al is intended to activate and expand gamma delta T lymphocytes for a variety of therapeutic uses; one of ordinary skill in the art would recognize that for activation of cells *in vitro* cells must be cultured for a suitable time period in the presence of the stimulus in order to achieve the desired result.

Regarding the duration of the culture period, the cell density before and during culture, and the concentration of the activator compounds (BrHPP and IL-2), in the field of cell culture such parameters are generally recognized to be result effective variables that would directly effect the final gamma delta T lymphocyte population within the sample; such parameters would routinely be optimized by one of

ordinary skill in the art. The length of time the cells are maintained in culture directly affects the final gamma delta T lymphocyte concentration within the sample. A longer culture period would ensure a higher number and percentage of gamma delta T lymphocytes, as the gamma delta T lymphocytes would have been exposed to the activator compounds for a greater length of time. One of ordinary skill in the art would have been motivated to manipulate the length of the culture based on the desired proportion of gamma delta T lymphocytes in the sample. For example, a longer culture period of 2-3 weeks would allow for a greater proportion of gamma delta T lymphocytes, which may be desirable for *in vitro* studies, where a more pure population of gamma delta T lymphocytes are desired; alternatively a shorter culture period of several days to 1 or 2 weeks would be desirable when a lower proportion of gamma delta T lymphocytes are required, which may be desired for reinfusion of the cells into a subject, or merely to save time and energy (Claims 27 & 28). The cell density of the initial cell culture, as well as the density throughout the culture period, directly affect the cells' ability to grow and divide; thus it would be well within the purview of one of ordinary skill in the art to initiate and maintain the culture at appropriate cell concentrations for the needs of the culture; the exact concentrations may vary based on how often the cell are passaged and how long the culture is to be maintained (Claims 21 & 24). Finally, the concentration of the activator compounds, both BrHPP and IL-2, would also directly effect the rate of proliferation and expansion of the gamma delta T lymphocytes in the culture. For example, Belmant et al teach the concentration of the IL-2 to be "in a proportion suitable to bring about lymphocyte growth" (See Belmant et al, col. 12, ln 5-7); therefore, while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL, manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art, again based on the desired activation rate of the cells as well as how often the cells are passaged (Claim 33). Generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical or produces unexpected results. Where the general conditions of a claim are

disclosed by the prior art it is not inventive to discover the optimum or workable ranges by routine experimentation, See *In re Aller,* 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

It then naturally follows that because all of the above discussed variables directly effect the final gamma delta T lymphocyte cell count and concentration in the final composition, it would have been within the purview of one of ordinary skill in the art to optimize any and each of the variables so as to produce a composition with any desired cell concentration and cell count, including at least 80% gamma delta T lymphocytes and at least 100 million cells. One would have been motivated to create a large cell population with a substantially high proportion of gamma delta T lymphocytes for use in *in vitro* studies on gamma delta T lymphocytes, or for reinfusion of the cells *in vivo* for treatment of various diseases, as disclosed by Belmant et al. One would have expected success optimizing the cell count and gamma delta T lymphocyte cell proportion because these variables are directly controlled by the culture steps and parameters discussed above; it would be well within the purview of one of ordinary skill in the art to optimize the culture conditions (extend culture time period, adjust time between passages, adjust concentration of activation agents) in order to create a gamma delta T lymphocyte composition with the desired cell numbers and proportions (Claim 34).

Still further, regarding the cytokines used in the experiments of Belmant et al, while Belmant et al added IL-2 to their media, it would further have been obvious to one of ordinary skill in the art at the time the invention was made to alternatively add IL-15 to the culture medium. In support see Garcia et al; Garcia et al teach that specific cytokines, including IL-2 and IL-15 are known to enhance gamma delta T lymphocyte activation in the presence of an activator of gamma delta T cells (See Garcia et al, Pg. 4324 & Fig. 2). Garcia et al unexpectedly found IL-15 to be a more potent induces of gamma delta T cell proliferation than IL-2 (See Garcia et al, Pg. 4324, col. 2). Therefore, it would have been well within the purview of one of ordinary skill in the art to alternatively utilize IL-15 in place of, or in addition to, the IL-2 used by Belmant et al (Claims 1 & 32). One of ordinary skill in the art would be motivated to

substitute IL-15 for IL-2 in the method of Belmant et al because Garcia et al teach IL-15 is more potent than IL-2 in enhancing gamma delta T cell proliferation. One would expect success based on the experimental findings of Garcia et al, which show successful activation and proliferation of gamma delta T cells in the presence of IL-15 and an activator compound. Regarding the concentration of IL-15 used, Garcia et al use of up to 100 ng/mL of IL-15, but report the effect of IL-15 on gamma delta T cell proliferation was dose dependent (See Garcia et al, Pg. 4324, col. 2). Therefore, while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL, manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art, again based on the desired activation rate of the cells as well as how often the cells are passaged (Claim 33).

Finally, while Belmant et al teaches the gamma delta T lymphocytes can be from a blood sample or blood extract, they do not specifically teach separating whole blood by cytapheresis prior to the culture method described above, nor do they teach using previous frozen samples of blood. However, at the time the invention was made it would have been well within the purview of one of ordinary skill in the art to obtain a blood sample at a first point in time, separate the whole blood sample by cytapheresis into individual components, freeze the individual components by appropriate means known in the art, and then at later time, thaw the desired platelet component for use in the method of Belmant et al (Claims 23 and 25). In support see Valeri; Valeri teaches the basic protocols for obtaining, separating and storing blood. Valeri teaches cytapheresis allows separation of specific components of blood from whole blood (See Valeri, Pg. 1, col. 1). Separating the platelets via 'plateletpheresis' removes the majority of red blood cells and plasma components, thereby increasing the proportion of gamma delta T lymphocytes present in the initial cell sample, which is desirable in the method of Belmant et al, as it would ensure a higher proportion of gamma delta T lymphocytes for culture and expansion, resulting in a greater end cell count. Additionally, Valeri teaches methods for frozen storage of separated platelets (See Valeri, Pg 297), which is desirable when the blood sample must be stored for a period of greater than 4 hours prior to use in the

method of Belmant et al (See Valeri, Pg. 6, col. 1). One would expect success separating the desired platelet component via cytapheresis and storing the separated components in a frozen state for future use, because such methods and procedures are common in the art of hematology, as demonstrated by the teachings of Valeri.

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 21, 23-25, 27-34 and 51-52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Espinosa et al (Journal of Biological Chemistry, 2001), in view of Garcia et al (Journal of Immunology, 1998) and Valeri (Blood Banking and the Use of Frozen Blood Products, 1976).

Applicants' claims are directed to a method for preparing a gamma delta T lymphocyte composition comprising culturing a biological preparation, comprising a blood sample or a cytapheresis sample, comprising at least 50 million mononuclear cells in the presence of (i) a synthetic activator compound of gamma delta T lymphocytes, selected from the group consisting of phosphohalohydrins, phosphoepoxides, pyrophosphates, biphosphonates, and bisphosphophonates; and (ii) a cytokine, selected from IL-2 or IL-15; and maintaining the cells at a density less than about 5×10^6 cells/mL during said culturing step. Claim 23 requires the sample to be from cytapheresis. Claim 24 requires the biological preparation to comprise more than 10×10^7 cells. Claim 25 requires the biological preparation to have previously been frozen. Claim 27 requires the cells to be cultured for greater than or equal to 10 days, claims 28 requires the culture period to be 10 to 25 days. Claims 29-31 require the synthetic activator to be BrHPP. Claim 32 requires the cytokine to be IL-2. Claim 33 requires the cytokine to be used at a concentration of about 150 U/mL to about 500 U/mL. Claim 34 requires the resulting gamma delta T lymphocyte population to comprise at least 80% gamma delta T lymphocytes and for the composition to comprise more than 100 million viable and functional gamma delta T cells. New claim 51 requires the

synthetic activator compound to be BrHPP, and the cytokine to be IL-2. New claim 52 requires the cytokine to be IL-15.

Espinosa et al sought to identify a synthetic activator of gamma delta T lymphocytes that has comparable immunostimulatory activity as natural phosphoantigens; Espinosa et al discovered BrHPP enabled immunostimulation of human gamma delta T lymphocytes (See Espinosa et al, abstract).

Espinosa et al first perform a control run using the known, natural phosphoantigen 3-formyl-1-butyl-pyrophosphate (3fbPP); peripheral blood lymphocytes were cultured at an initial concentration of 10^6 cells/mL in the presence of 10nM 3fbPP and 100 U/mL IL-2 for a 15 day period (See Espinosa et al, Pg. 18338, col. 1). Espinosa et al report significant expansion of the gamma delta T lymphocytes, including compositions comprising greater than 95% TCR Vδ2 positive cells (gamma delta T lymphocytes) (See Espinosa et al, Pg. 18338, col. 2).

Espinosa et al then perform an experimental run using several different concentrations (12.5, 25, 100 nM) of BrHPP as the activator instead of the natural 3fbPP (See Espinosa et al, Pg. 18340, col. 1-2 & Fig. 4). Espinosa et al do not specifically describe the culture conditions of the experimental run, while they do state that peripheral blood cells were used, they are silent on the initial cell count, the length of the culture period, and whether or not IL-2 was added to the culture. However, it appears the culture conditions for the experimental run were identical to the conditions of the control run: 10^6 cells/mL were present in initial culture as well as 100 U/mL of IL-2, and the culture was maintained for 15 days. One of ordinary skill in the art would assume that for results to be comparable between the immunostimulatory activity of the 3fbPP and BrHPP, the culture conditions were identical. Therefore, in the absence of evidence to the contrary, it is assumed Espinosa et al performed a method for activation of a gamma delta T lymphocyte composition comprising culturing peripheral blood lymphocytes (PBL) in the presence of BrHPP (a synthetic activator of gamma delta T lymphocytes) and IL-2.

However, even if the culture conditions described for the control run were not duplicated in the experimental run, it would have been well within the purview of one of ordinary skill in the art to optimize the duration of the culture period, the cell density before and during culture, and the concentration of the activator compounds (BrHPP and IL-2), in the field of cell culture such parameters are generally recognized to be result effective variables that would directly effect the final gamma delta T lymphocyte population within the sample; such parameters would routinely be optimized by one of ordinary skill in the art. The length of time the cells are maintained in culture directly affects the final gamma delta T lymphocyte concentration within the sample. A longer culture period would ensure a higher number and percentage of gamma delta T lymphocytes, as the gamma delta T lymphocytes would have been exposed to the activator compounds for a greater length of time. One of ordinary skill in the art would have been motivated to manipulate the length of the culture based on the desired proportion of gamma delta T lymphocytes in the sample. For example, a longer culture period of 2-3 weeks would allow for a greater proportion of gamma delta T lymphocytes, which may be desirable for *in vitro* studies, where a more pure population of gamma delta T lymphocytes are desired; alternatively a shorter culture period of several days to 1 or 2 weeks would be desirable when a lower proportion of gamma delta T lymphocytes are required, which may be desired for reinfusion of the cells into a subject, or merely to save time and energy (Claims 27 & 28). The cell density of the initial cell culture, as well as the density throughout the culture period, directly affect the cells' ability to grow and divide; thus it would be well within the purview of one of ordinary skill in the art to initiate and maintain the culture at appropriate cell concentrations for the needs of the culture; the exact concentrations may vary based on how often the cell are passaged and how long the culture is to be maintained (Claims 21, 24 & 26). Finally, the concentration of the activator compounds, both BrHPP and IL-2, would also directly effect the rate of proliferation and expansion of the gamma delta T lymphocytes in the culture. For example, Espinosa et al teach the concentration of the IL-2 to be 100 U/mL (See Espinosa et al, Pg. 18338, col. 1); however,

while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL, manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art, again based on the desired activation rate of the cells as well as how often the cells are passaged (Claim 33). Generally, differences in concentration will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration is critical or produces unexpected results. Where the general conditions of a claim are disclosed by the prior art it is not inventive to discover the optimum or workable ranges by routine experimentation, See *In re Aller,* 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955).

It then naturally follows that because all of the above discussed variables directly effect the final gamma delta T lymphocyte cell count and concentration in the final composition, it would have been within the purview of one of ordinary skill in the art to optimize any and each of the variables so as to produce a composition with any desired cell concentration and cell count, including at least 80% gamma delta T lymphocytes and at least 100 million cells. In the instant reference Espinosa et al report a final gamma delta T lymphocyte population which comprises approximately 63% of total lymphocytes (See Espinosa et al, Fig. 4a); alternatively, Espinosa et al teach the concentration of BrHPP directly effects the final gamma delta T lymphocyte count (See Pg. 18340, col. 2 & Fig. 4B). Thus one would have expected success optimizing the cell count and gamma delta T lymphocyte cell proportion because these variables are directly controlled by the culture steps and parameters discussed above, particularly the concentration of BrHPP; it would be well within the purview of one of ordinary skill in the art to optimize the culture conditions (extend culture time period, adjust time between passages, adjust concentration of activation agents) in order to create a gamma delta T lymphocyte composition with the desired cell numbers and proportions (Claim 34).

Still further, regarding the cytokines used in the experiments of Espinosa et al, while Espinosa et al added IL-2 to their media, it would further have been obvious to one of ordinary skill in the art at the

time the invention was made to alternatively add IL-15 to the culture medium. In support see Garcia et al; Garcia et al teach that specific cytokines, including IL-2 and IL-15 are known to enhance gamma delta T lymphocyte activation in the presence of an activator of gamma delta T cells (See Garcia et al, Pg. 4324 & Fig. 2). Garcia et al unexpectedly found IL-15 to be a more potent induces of gamma delta T cell proliferation than IL-2 (See Garcia et al, Pg. 4324, col. 2). Therefore, it would have been well within the purview of one of ordinary skill in the art to alternatively utilize IL-15 in place of, or in addition to, the IL-2 used by Espinosa et al (Claims 1 & 32). One of ordinary skill in the art would be motivated to substitute IL-15 for IL-2 in the method of Espinosa et al because Garcia et al teach IL-15 is more potent than IL-2 in enhancing gamma delta T cell proliferation. One would expect success based on the experimental findings of Garcia et al, which show successful activation and proliferation of gamma delta T cells in the presence of IL-15 and an activator compound. Regarding the concentration of IL-15 used, Garcia et al use of up to 100 ng/mL of IL-15, but report the effect of IL-15 on gamma delta T cell proliferation was dose dependent (See Garcia et al, Pg. 4324, col. 2). Therefore, as discussed above, while applicant specifically claims a cytokine concentration in the range of 150 U/mL to 500 U/mL, manipulation and optimization within this range would be well within the purview of one of ordinary skill in the art based on the desired activation and proliferation rate of the cells as well as how often the cells are passaged (Claim 33).

Finally, while Espinosa et al teach use of peripheral blood lymphocytes, they do not specifically teach separating whole blood by cytapheresis prior to the culture method described above, nor do they teach using previous frozen samples of blood. However, at the time the invention was made it would have been well within the purview of one of ordinary skill in the art to obtain a blood sample at a first point in time, separate the whole blood sample by cytapheresis into individual components, freeze the individual components by appropriate means known in the art, and then at later time, thaw the desired platelet component for use in the method of Espinosa et al (Claims 23 and 25). In support see Valeri;

Valeri teaches the basic protocols for obtaining, separating and storing blood. Valeri teaches cytapheresis allows separation of specific components of blood from whole blood (See Valeri, Pg. 1, col. 1). Separating the platelets via 'plateletpheresis' removes the majority of red blood cells and plasma components, thereby increasing the proportion of gamma delta T lymphocytes present in the initial cell sample, which is desirable in the method of Espinosa et al, as it would ensure a higher proportion of gamma delta T lymphocytes for culture and expansion, resulting in a greater end cell count. Additionally, Valeri teaches methods for frozen storage of separated platelets (See Valeri, Pg 297), which is desirable when the blood sample must be stored for a period of greater than 4 hours prior to use in the method of Espinosa et al (See Valeri, Pg. 6, col. 1). One would expect success separating the desired platelet component via cytapheresis and storing the separated components in a frozen state for future use, because such methods and procedures are common in the art of hematology, as demonstrated by the teachings of Valeri.

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Allison M. Ford whose telephone number is 571-272-2936. The examiner can normally be reached on 7:30-5 M-Th, alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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